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VAM AND DARK SEPTATE ENDOPHYTIC FUNGI IN A TROPICAL DRY DECIDUOUS FOREST OF CENTRAL INDIA.

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ABSTRACT

Vesicular arbuscular mycorrhizal colonization and spore density was studied in 12 forest plants collected from Tropical Dry Deciduous Forests of Sagar (M. P.). Root and rhizosphere soil samples collected from forest plant species and analysed for quantitative and qualitative study of VAM Fungi and DSE. VAM spores were isolated and their quantitative estimation was done. We observed three mycorrhizal types including Arum-type, Paris-type, and an intermediate type among the plants. Another type of potentially beneficial fungi associated with roots of nine species was also observed, namely, dark septate endophytic fungi (DSEF). Of the 12 sample plants examined, nine species were co occurred by the two target fungi (Vesicular Arbuscular mycorrhizal fungi (VAMF) and DSEF). Maximum VAM colonization was observed in *Tectona grandis* and minimum in *Albizia lebbek* Benth. Based on this investigation, we speculated that the DSEF are ubiquitous in forest ecosystems and can co-occur with VAMF in forest plants, functioning much like VAM fungi. Further studies will be required to elucidate interactional mechanisms with VAMF and the mechanisms operating in forest ecosystem.

Keywords, Vesicular Arbuscular mycorrhizal fungi, mycorrhiza type, Dark Septate Endophytes

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Introduction

Vesicular arbuscular mycorrhizal fungi form mutualistic associations with the roots of terrestrial plants, providing them with phosphorus and other nutrients in exchange for photosynthate (Allen, 1991; Smith and Read, 1997). It is estimated that about 87% of terrestrial plants form mycorrhizal associations (Stoyke and Currah, 1993). Mycorrhizal fungi are well adopted for nutrient acquisition, their small size allowing them to act as microscopic pipelines that can transport carbon and minerals to and fro from the plant(Barrow, 2004),and under stressed environment (Barrow and Aaltonen, 2001; Griffin, 1979).

In addition to mycorrhiza, the roots of plants may get frequently colonized by fungi with dematiaceous septate hyphae which are often sterile in culture conditions (Newsham, 1999). Such fungi are collectively called as dark septate endophytes (DSE). DSE refer to a miscellaneous group of ascomycetous and anamorphic fungi. Once DSE colonized the roots of the plants, they form characterized inter and intracellular structures including a superficial net of hyphae, penetration into the cortical layer, microsclerotia and occasionally, a partial mantle (Jumpponen and Trappe, 1998). DSE can be easily distinguished from AM fungal hyphae by their dark red brown to dark brown colour, thick lateral wall and

frequent septa. So far DSE have been reported mostly from arctic (Jumpponen and Trappe, 1998), alpine environments (Read and Haselwandter, 1981), acidic organic soils (Wurjburger and Blensoe, 2001), neotropical cloud forests (Rains *et al*., 2003), peat bogs and fen meadows (Fuchs and Haseleandter, 2004), grassland ecosystem (Mathew and Malathy, 2007).occurrence of DSE in tropical forests has not been well documented.

Materials and Methods

The present study was carried out in tropical dry deciduous forests around Sagar (M. P.) in central India (23^0 o5'-24^0 25' N and 78^0 10'-79^0 15'). Root and rhizosphere soil samples were collected from dominant plant species. Root samples were processed and stained following Phillips and Haymann (1970) and Kormainik *et al*., (1980). root Samples were also stained with 0.5%acid fuchsin as for DSE. Per cent root colonization was observed taking 100 radomly selected root pieces. Qualitative characteristics of VAM fungi i.e. external hyphae, vesicles, arbuscules paris coil and endospores were observed in stained root samples. VAM spore extraction was done by wet sieving and decanting method (Gerdemann and Nicolson, 1963), and spore count was followed after Gour and Adholeya, 1994. Identification of VAM fungi was done on current descriptions and identification manuals (Schenk and Perez, 1990; Mehrotra and Baizal, 1994; http-://vam.caf.wvu.edu./myc-info/taxonomy/species.htm). Species name codes of VAM fungi were followed after Perez and Schenk (1990).

Results and Discussion

Among the 12 plants in this experiment, we found different plant species formed different mycorrhizal morphology, consisting of mainly three VAM types of Arum type, Paris type, and intermediate type. The Arum type formed extensive intercellular hyphae. The Paris type was characterized by the absence of intercellular hyphae and replaced by extensive intracellular hyphal coils (Fig.1 (a)). Moreover, if two morphological structures were represented in the same root system, then it was termed as intermediate type (Fig.1 (b)). The mycorrhizal types of the target plants are described in Table 1. All the species were colonized by VAMF, although *Miliusa tomentosa* was not observed to have vesicles, *Pithecelobium dulce Benth, Holopetilia integifolia, Acacia leucoploea, Diospyros melanoxylon Tectona grandis* were not observed to have arbuscules. Most of the studied species had Vesicular arbuscular colonization, with only low vesicular arbuscular colonization of *Albizia lebbek* Benth. The spore density (expressed as per 25 g dry soil) under the canopy differed greatly among plants, ranging from 32 to 91 (Table 1). In this investigation, plant species formed different AM morphology, suggesting that the morphology of AM was depended on the genotype of host plant (Ahulu *et al*., 2007). Coexistence of DSEF and VAMF was detected in nine investigated plants , although some species had no DSEF colonizationand microsclerotia such as in *Holopetilia integifolia, Diospyros melanoxylon* , *Mitragyna parviflora* Roxb. and maximum VAM colonization was observed in *Tectona grandis* and minimum in *Albizia lebbek* Benth. All 12 species had VAMF and nine had formed microsclerotium.

Fig. 1 .Mycorrhizal types

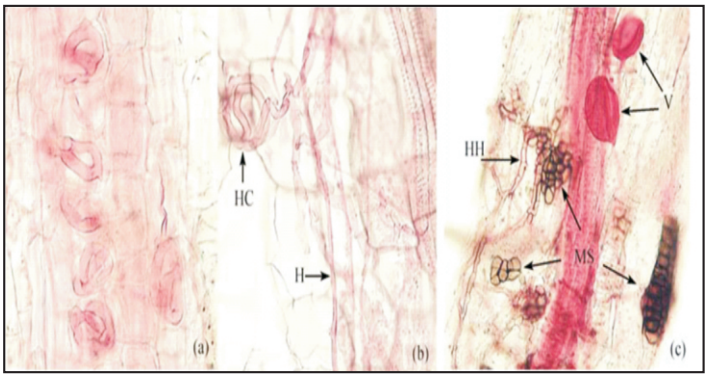

Fig.1. (a), (b) and (c) represent Paris-type of *Acacia leucoploea*, intermediate type of *Diospyros melanoxylon*, and coexistence of VAMF and DSEF in *Diospyros melanoxylon*. respectively. H, HC, V, HH and MS represent hyphae of AMF, hyphal coils of AMF, vesicles of AMF, hyaline hypha of DSEF and microsclerotium, respectively.

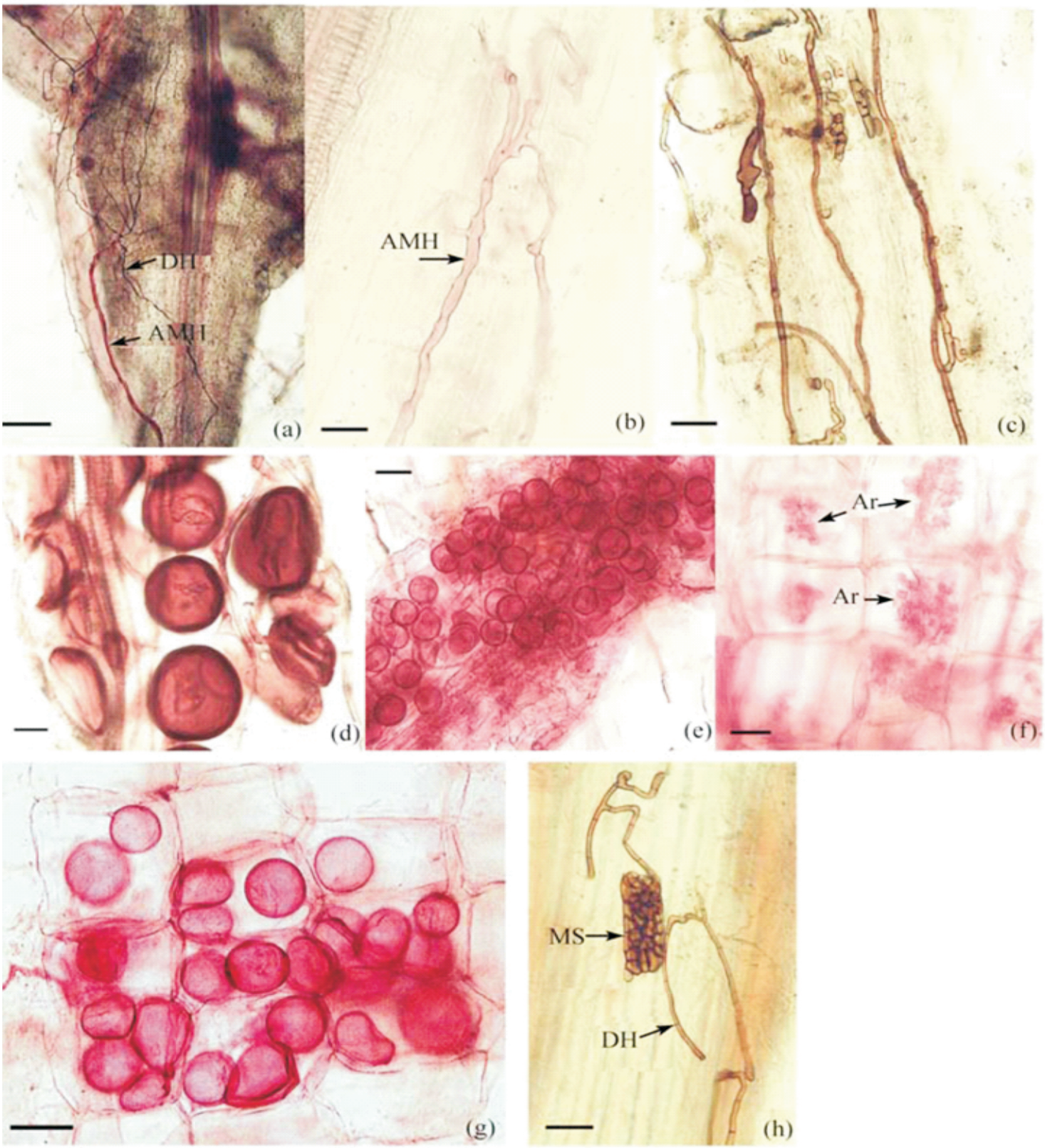

Fig. 2. Colonization by AMF and DSEF in tissues

Table 1. Status of different structures and spore density of AMF and DSEF tissues in the forest plants.

Plants Name	Mycorrhiza types	Hyphae/%	Vesicle/%	Arbuscule/%	DSE/%	MS/%	Total VAM ／%	Spore Density/ (spore/25 g soil)
Pithecelobium dulce Benth.	I	2	28	0	6	3	46	59
Holopetilia integifolia	A	56	57	0	0	0	37	52
Miliusa tomentosa	I	78	0	2	23	4	28	38
Albizia lebbek Benth	I	89	56	5	4	2	13	32
Lagerstroemia parviflora Roxb.	I	53	41	24	44	21	44	57
Santalum album	P	76	34	2	17	6	16	34
Acacia leucoploea	P	40	79	0	4	3	48	49
Diospyros melanoxylon	I	75	88	0	0	0	76	74
Mitragyna parviflora Roxb.	A	32	34	0	0	0	42	52
Tectona grandis	P	79	12	0	12	9	86	91
Butea monosperma	P	42	23	9	35	13	82	88
Cassia fistula Linn.	I	13	16	3	16	6	79	80

VAMF plays an important role in plant survival and community stability of vegetation in natural ecosystems (Gange *et al*., 1993; Francis and Read, 1994; Hartnett and Wilson, 2002; Moraes *et al*., 2004). It is well known that VAMF can improve plant nutrient uptake (Tarafdar and Kumar, 1996), water use efficiency (Auge and Stodola, 1990), and resistance to abiotic stress under certain conditions (Brundrett, 1991). The present study indicated that VAMF was formed in the roots of various plants in tropical dry deciduous forest, which demonstrated that these associations might be one of the valid counter measures of healthy plants to the forest ecosystems. Plant community structure and VAMF distribution and colonization status might be used to monitor forest and soil degradation. Several studies on VAMF have been conducted in recent years in forest ecosystems (Collier *et al*., 2003; Ferrol *et al*., 2004; Alarcón and Cuenca, 2005).The variation in spore density and colonization of VAMF associated with different host plant species may be generated by a variety of potential mechanisms, including biological characteristics of rhizosphere under host species, variation in host species, mycorrhizal dependency, host plant-mediated alteration of the soil microenvironment,or other unknown host plant traits, as described by Lorgio *et al*. (1999) and Eom *et al*., 2000. In our present study, no significant correlation was observed between VAM F colonization and spore density. However, some researchers found a positive relationship between VAMF colonization and spore density (Ebbers *et al*., 1987; Sigüenza *et al*., 1996), whereas others found a negative relationship (Fontenla *et al*., 1998). We found that DSE infected nine tested plants and

cooccurred with VAMF in the plant roots. The results indicated that the common and beneficial species of forest plants could easily form AM and DSEF tissues. Mandyam and Jumpponen (2005) speculated that DSE fungi would be prevalent in various habitats and colonize a substantial proportion of the species present in mixed plant communities. This group of fungi cannot be overlooked while assessing the fungal communities of any ecosystem, as their abundance may equal or even exceed that of the VAM fungi. Although different opinions exist regarding whether DSEF should be treated as mycorrhizal fungi or not, there seems to be an agreement that some members

play an important role in soil ecosystems in some cases, with DSEF functioning much like mycorrhizal fungi (Jumpponen and Trappe, 1998; Jumpponen, 2001). In the present study, most of the plants have hyphal and vesicular colonization. This result is due to the mycelium and vesicles, which are the primary structures existing for months or years. Generally, arbuscules begin to form approximately two days after root penetration and begin to collapse after a few days (Smith, 1995), DSE tissue may be AMF competing with DSEF for the econiche. The microsclerotia of some species of DSE had some special storage substances and may function as propagules (Yu *et al*., 2001). Therefore, we suggest that the function and growth characteristics of microsclerotium may be similar to the vesicular and arbuscular. In general, the DSEF function and the interaction on AMF are less well known. Our study indicates that the plants in tropical dry deciduous forest may establish a good symbiosis with DSEF, and the symbionts may depend on each other for survival in these forest environments. Moreover, further studies will be required to elucidate the interactional mechanisms with AMF and the operating mechanisms in forest ecosystems.

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